Development of the Murine and Human Immune System: Differential Effects of Immunotoxicants Depend on Time of Exposure

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Fetal and early postnatal life represent critical periods in vertebrate immune system development. Disruption of such development by perinatal immunotoxic chemical exposure has been widely described in experimental animal models. The resultant inhibited postnatal immune responses in such animals are often more dramatic and persistent than those after exposure during adult life. Further, recent reports suggest that prenatal exposure to immunotoxicants may exacerbate postnatal aberrant immune responses (e.g., hypersensitivity disorders and autoimmune disease) in genetically predisposed rodents. Limited information is available regarding the possibility of inhibited postnatal immune capacity in humans as a result of developmental immunotoxicant exposure. The multifactorial nature of hypersensitivity and autoimmune responses will further complicate the elucidation of possible relationships between chemical exposure during ontogeny of the human immune system and immune-mediated disease later in life. Taken together, however, the available animal data suggest the potential for altered postnatal immune function in humans exposed to immunotoxicants (e.g., environmental chemicals and therapeutic agents) during fetal and/or early postnatal life. *Key words*: autoimmune disease, developmental immunotoxicity, diethylstilbestrol, immune development, TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, prenatal, therapeutic immunosuppressant. — *Environ Health Perspect* 108(suppl 3):463–473 (2000).
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Establishment of the vertebrate immune system requires a sequential series of carefully timed and coordinated developmental events that begin early in embryonic/fetal life and continue through the early postnatal period. Perturbation or abrogation of this developmental sequence of events can lead to immune dysfunctions that may be life threatening. Defects in the development of the immune system due to heritable changes in the lymphoid elements provide clinical and experimental examples of the devastating consequences of impaired immune development.

Potential functional defects caused by exposure to toxic agents during development may range from life-threatening suppression of vital components of the immune system to altered or poorly regulated responses that can be debilitating. For example, studies in laboratory rodents indicate that exposure to immunotoxicants (e.g., environmental chemicals, drugs, and ionizing radiation) during this critical time of immune system development may produce persistent, and in some cases dramatic, effects on postnatal immune function. For certain chemical agents {e.g., chlordane; benzo[*a*]pyrene (B[a]P); and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)}, perinatal exposure has been associated with what appears to be lifelong immunosuppression. For some of the same and other chemical agents (e.g., therapeutic or environmental estrogens and TCDD), there is evidence that exposure early in life may exacerbate or induce autoimmune responses in genetically predisposed individuals. The full extent of the immunological effects resulting from exposure to environmental agents during immune system development is not yet known; however, the potential for serious consequences is obvious.

Developmental immunotoxicology as a discipline is in its infancy, with much remaining to be learned. Indeed, limited human data are available regarding the spectrum of potential postnatal immune consequences that may result from gestational and/or early postnatal exposure to individual or mixtures of immunotoxic compounds. We describe the development of the immune system and compare the similarities and differences of this development in humans and laboratory animals; review the evidence that the developing immune system is sensitive to perturbation by chemical and physical agents and that consequent agent-induced immune dysfunction may continue through life; and identify the gaps in the developmental immunotoxicology knowledge base.

Development of the Immune System

Cellular and humoral immune responses in the neonate differ both qualitatively and quantitatively from those of the adult. These differences are an anticipated result of lower numbers and/or decreased functional capacity of leukocytes [e.g., T and B lymphocytes, natural killer (NK) cells, and myeloid-lineage cells] present in the early postnatal immune system (*1*). The postnatal immune system is, in turn, the product of a series of highly regulated developmental events that include sequential waves of hematopoietic cell production, cellular migrations through hematopoietic organs, cell–cell interactions within these organs under microenvironmental influences, highly specific cytodifferentiational steps, and final maturation, including the acquisition of definitive functional properties (*2,3*). Most available data regarding ontogenesis of the vertebrate immune system are derived from human and rodent studies, and demonstrate multiple switching of hematopoietic compartments during development (*4–8*). Relatively limited data are available in species other than humans and rodents, but those that exist suggest similar patterns of immune development. However, the stage of development achieved at the time of birth, and, thus, the degree of immunocompetence in early postnatal life, is variable from species to species (*9*).

Early in vertebrate development (e.g., at approximately 24 hr in the chicken), mesodermal-derived hemangioblasts give rise to the earliest progenitors of all blood cells. These pluripotent stem cells are first detected in the area vasculosa, a plexus of vessels formed on the surface of the yolk sac before the establishment of closed circulation (*10*). Shortly thereafter, pluripotent stem cells appear in blood islets within the yolk sac (*5*), and, at approximately day 9 of gestation in the mouse, migrate via the bloodstream to the fetal liver and spleen. The liver and, to a lesser degree, the spleen then carry the burden of hematopoiesis until shortly after birth (*8*).

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Fetal liver hematopoiesis is first detectable at approximately day 10 of gestation in mice and at about the sixth week of gestation in humans (*4,8*). The mouse spleen actively contributes to hematopoiesis from approximately day 15 of gestation until several weeks after birth, at which time erythropoietic elements predominate (*6,8*). Although the spleen never completely loses hematopoietic function in the mouse, this organ has largely ceased hematopoiesis in humans by the time of birth (but may regain hematopoietic function in abnormal situations) (*11*).

Perinatally in both mice and humans, pluripotent stem cells in the liver migrate to bone marrow as the liver becomes increasingly devoted to metabolic function. The vascular mesenchyme within the bone marrow forms a supporting reticular network by gestational days 17–18 in mice and by week 20 in humans upon which the migrating pluripotent stem cells can seed and proliferate (*12*). Waves of granulopoietic activity dominate murine marrow hematopoietic function from about day 19 of gestation until approximately 3 months after birth. Limited erythropoietic activity begins in mouse bone marrow after birth and in humans well before birth (*8*). A limited number of B cell progenitors can be detected in mouse bone marrow on day 19 of gestation; they increase in number until about 2–4 months of age, at which time they constitute approximately 20% of the nucleated cell population. A limited number of T lymphocytes can be detected in murine bone marrow at birth, and they slowly increase to plateau at about 2–4 months of age (*6*). In both mice and humans, the bone marrow then serves as the primary site of hematopoiesis throughout postnatal life.

The epithelial thymic rudiment forms at gestational days 9–10 in the mouse (*6,13*), after which colonization of the thymus by precursor T cells from the fetal liver begins on days 10–11 (*13,14*). These thymocytes are initially double negative with respect to CD4 and CD8 cell-surface antigens, then develop sequentially to immature $CD8^+$ single positive and $CD4^+CD8^+$ (double positive) stages, and finally mature into $CD4^+$ or $CD8^+$ thymocytes that migrate to the periphery as functional T-helper and cytotoxic T lymphocytes, respectively. Rearrangement of T-cell receptor (*TCR*) gene segments occurs during this maturation, resulting in appearance of double-positive thymocytes expressing surface TCR (*15,16*). The TCR complex that includes a product of the *TCR* γ-gene is present on fetal thymocytes by days 14–15 of gestation (*17,18*). At approximately day 17 of fetal mouse development, the TCR αβ-heterodimer is expressed on the majority of thymocytes (*19*). Anti-CD3 monoclonal antibody is able to activate day-16 fetal thymocytes, however (*20*), indicating that CD3-bearing day-16 fetal thymocytes express a functional TCR before TCR-αβ structures are expressed (*19*).

In contrast to the mouse, where thymic colonization occurs in the latter half of gestation, thymic colonization and early T-cell development occur in the first trimester of fetal development in humans. Bloodborne stem cells enter the developing thymus at approximately 7 weeks of gestation, with subsequent differentiation occurring rapidly and following a pattern similar to that seen in the mouse. The majority of these thymocytes express TCR of the γδ type at approximately 9.5 weeks of gestation and of the αβ type by about 10 weeks of gestation (*1,21*). Presently, it is unclear whether the diversity of the T cell repertoire in rodents and humans is lower at birth than in adulthood. Further, the capacity of such T cells in rodents and humans at birth to provide help for immunoglobulin secretion is limited mainly to IgM production. This may in part be due to reduced ability of T cells from neonates to produce cytokines [e.g., interleukin (IL)-2, IL-4, and interferon-γ] required for activation of T-helper (Th) cells, as well as macrophages, NK cells, and cytotoxic T lymphocytes (*1*).

B lymphopoiesis can be identified in mouse fetal liver by days 12–13 of gestation (*22*). Pre-B cells can be found in the fetal liver of mice by day 14, and B lymphocytes, which are for the most part restricted to IgM expression, can be found by days 16–17. The general scheme of early B-cell development in mice includes *a*) a series of well characterized molecular steps involving the assembly of gene segments encoding the immunoglobulin molecule, *b*) heavy chain D–J rearrangements to yield progenitor B cells, and *c*) light chain V–J rearrangements to generate B cells expressing complete cell-surface Ig (*23–25*). These molecular events, which have been demonstrated in both fetal liver and neonatal spleen in the mouse, appear identical to those occurring throughout life in adult bone marrow. However, neonatal mouse B lymphopoiesis differs from the adult mouse in the level of expression of certain enzymes [e.g., terminal deoxynucleotidyl transferase, precursor lymphocyte-regulated myosin-like light chain, and in major histocompatibility complex (MHC) class II surface antigen expression]. Hayakawa et al. (*22*) suggested that such fetal differences from adult B lymphopoiesis during development may be related to differences in susceptibility to tolerance.

IgM synthesis can be detected by approximately weeks 10–12 of gestation in human fetal liver. Serum IgM concentrations are only approximately 10% of adult levels at birth, and do not reach adult levels until 1–2 years of age. Similarly, concentrations of IgG in serum do not reach adult levels for 4–6 years after birth. Further, human neonates younger than 2 years age are not able to mount humoral responses to some antigens (e.g., carbohydrate antigens), an observation that may in part explain the increased susceptibility of infants to certain bacterial infections (e.g., *Streptococcus pnenumoniae*) (*1*).

Consequences of Immunotoxicant Exposure

Prenatal Exposure

Organogenesis of the immune system occurs during the prenatal and, to a lesser extent, early postnatal periods of mammalian development. As might be predicted from this, the perinatal period is a time of high sensitivity to immunotoxicants that cross the placenta or enter the neonate via lactation. Postnatal immunotoxic consequences from such chemical exposure during the initial establishment of the immune organs may be both more severe and more persistent than those that occur in adult animals exposed at similar levels [reviewed by Holladay and Luster (*26*)]. Indeed, for a growing list of chemical agents, the exposure of pregnant animals to immunotoxicants at levels that produce limited transient effects in adults produces long-lasting or even permanent immune deficits in the offspring.

Chemical agents that cause developmental immunotoxicity in rodents are diverse and include halogenated aromatic hydrocarbons (HAHs), polycyclic aromatic hydrocarbons (PAHs), hormonal substances, therapeutic agents, heavy metals and mycotoxins are summarized as follows [modified from Holladay and Luster (*26*)]:

- polycyclic halogenated hydrocarbons: TCDD, PCBs, and PBBs
- polycyclic aromatic hydrocarbons: B[*a*]p; methylcholanthrene; and 7,12-dimethylbenz[*a*]anthracene
- pesticides: hexachlorocyclohexane, chlordane, diazinon, DDT, and carbofuran
- fungicides: hexachlorobenzene
- heavy metals: methyl mercury, lead, and cadmium
- hormonal substances: estrogens/diethylstilbestrol (DES), testosterone, and cortisone
- therapeutic agents: acyclovir, busulfan, cyclophosphamide, and cyclosporin A
- mycotoxins: T-2 toxin
- irradiation: X rays.

With the exception of a limited database in humans who were exposed to therapeutic immunosuppressive drugs or to the nonsteroidal estrogen DES during gestation, details regarding postnatal consequences from early human exposure to known adult immunotoxicants remain largely unavailable.

A relatively new and growing concern is that such early human exposure to immunotoxic compounds may not only cause postnatal immunosuppression, but may also result in increased expression of aberrant immune responses (e.g., hypersensitivity and autoimmune disease).

HAHs. Considerable effort has been devoted to understanding the effects of PAHs on the developing immune system. The most studied compound in this group of chemicals for immunotoxicity is TCDD. Much of the immunotoxicity of TCDD and congeners of TCDD (halogenated biphenyls, chlorinated dibenzofurans, and other chlorinated dibenzodioxins) is directly proportional to the binding affinity of the individual congeners to the aryl hydrocarbon (*Ah*) receptor (*27*). For this reason, many of these agents produce a similar pattern of immunotoxic responses in animals (*28*). TCDD in particular produces profound thymic atrophy in all species examined [reviewed by Vos and Luster (*29*)]. Further, when administered during the maturational development of the immune system, TCDD causes more persistent immunosuppression than if given in adult life (*30*). Thymic atrophy in neonates and adults occurs after remarkably low-level exposure to TCDD and has been well characterized. TCDD crosses the mouse placenta relatively poorly [i.e., < 0.5% of an oral dose (*31*)]; however, single-dose or subacute exposure of pregnant mice to TCDD in the low milligram-per-kilogram range causes a highly significant involution of the fetal thymus as well as inhibition of thymocyte differentiation (*32*). Such exposure to TCDD results in inhibited T-cell responses lasting 8–10 weeks after birth in exposed mice (*33*). Other effects on cell-mediated immune function after pre- or perinatal TCDD exposure include suppression of T-cell mitogen responses (*34*), skin graft rejection times, graft-versus-host reactivity (*35*), and delayed hypersensitivity (*30,36–39*). In addition, perinatal TCDD exposure increases susceptibility to challenge with infectious agents or syngeneic tumor cells (*30*). Thus, the collective available data indicate that TCDD exposure during development of the immune system results in a relatively long-lasting postnatal impairment of cell-mediated immune function.

Polyhalogenated biphenyls [polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs)] represent a heterogeneous class of HAHs that have been associated with immunological effects in humans and animals (*40–42*). Reports of developmental immunotoxicity caused by PCB or PBB exposure vary from little or no observed toxicity to highly significant immune system alterations. For instance, when PBBs were administered during gestation in mice and rats, a variety of immune parameters including hematology, serum immunoglobulin and antibody titer, delayed hypersensitivity response, lymphoproliferative responses, and several macrophage functions were not affected in the offspring (*42*). These authors also found that calves inadvertently exposed to PBB *in utero* displayed no significant alterations in immune responses. When pregnant mice were dosed with 0.5 mg/kg/day of the PCB 3,4,5,3′,4′,5′-hexachlorobiphenyl (HCB) beginning at the day of implantation, very marginal effects on the developing immune system were again observed (*43*). Similarly, feeding pregnant mice throughout gestation and lactation with various combinations of lead and/or PCB resulted in negligible effects on the ability of the offspring to mount an immune response (*44*). However, when lead alone was administered throughout gestation and lactation, 35- to 45-day-old rats displayed reduced thymic weights and delayed hypersensitivity responses and inhibited lymphocyte proliferation (*45*). These reports indicate that certain PBBs and PCBs are not potent developmental immunotoxicants.

In contrast to the above results, significant alterations were reported in peripheral lymphocytes of 4-week-old piglets after perinatal exposure to a commercial mixture of PBB (Firemaster BP-6) (*46*). Four-week-old mice exposed prenatally to a commercial PCB mixture (Kanechlor 500) displayed T-helper activity reduced to 20% of control levels (*47*). In another report, *in utero* exposure of mice to PCB resulted in fetal thymic hypoplasia and inhibited lymphoid cell development in thymic organ culture (thymi taken from 14-day embryos) (*48*). Thus, published reports have varied considerably regarding the toxicity of halogenated biphenyls to the developing immune system. These different immunotoxicity outcomes may in part be the result of differences in structure of the congeners evaluated. In particular, for some toxic end points, PCBs with chlorines in the *para* positions and in at least one of the *meta* positions of each ring, but not in any *ortho* position, are the most toxic members of this chemical class (*49*). These PCBs, including 3,3′,4,4′-tetrachlorobiphenyl (TCB), 3,3′,4,4′,5-pentachlorobiphenyl (PeCB), and 3,4,5,3′,4′,5′-HCB, bind the *Ah* receptor with relatively high affinity (though with less avidity than TCDD) (*50*). Mono- and *ortho*-chlorinated biphenyls, in contrast, are weak *Ah* receptor ligands and thus do not produce *Ah*-receptor mediated immunotoxicity at levels comparable to congeners with higher receptor affinity. In support of *Ah* receptor binding affinity as a mechanism explaining divergent reports of PCB immunotoxicity, median effective dose (ED_{50}) values for inhibition of the developing bursa of Fabricius in chick embryos were 4 µg PeCB, 50 µg TCB, and 300 µg/kg egg HCB, whereas the mono-*ortho*-chlorinated analogs of TCB and PeCB were 1,000 times less potent inhibitors of bursal development than PeCB. When mouse thymi were taken from gestational day 15 mouse fetuses and treated *ex vivo* with TCDD or PCB in organ culture, inhibited thymocyte development again correlated with *Ah* receptor binding affinity. For example, PeCB was only one-tenth as potent ($EC_{50} = 2 \times 10^{-9}$ M) as TCDD ($EC_{50} = 2 \times 10^{-10}$ M) in inhibiting thymocyte development, whereas the potencies of HCB and TCB were approximately one-hundredth that of PeCB. No inhibition of thymocyte development was produced in this system by concentrations of mono-*ortho*-chlorinated PCB as high as 10^{-6} M (*50*).

There are reports that document developmental immunotoxicity of certain monocyclic and polycyclic halogenated pesticides and pesticide contaminants, including the fungicide hexachlorobenzene (*51*); the herbicide contaminant 3,3′,4,4′-tetrachloroazoxybenzene (*52*); and the insecticides hexachlorocyclohexane (*53*), diazinon, and carbofuran (*54*) and chlordane (*55*). Hexachlorobenzene exposure at 0.5 mg/kg/day throughout gestation resulted in long-lasting (into adulthood) depressed delayed-type hypersensitivity (DTH) responses and decreased numbers of splenic B cells in exposed mice *in utero* (*51*). When pregnant mice were exposed to higher levels of hexachlorobenzene, Barnett et al. (*51*) noted a significantly impaired mixed lymphocyte response. In contrast, rats exposed pre- and postnatally via their diet to approximately 4, 20, or 100 mg/kg hexachlorobenzene had significantly enhanced humoral immune function and DTH responses (*56*). The reasons for this different observation in rodents exposed to hexachlorobenzene is unknown but may be due to dose level. In this regard, when pregnant mice were exposed to 10 or 100 mg/kg body weight hexachlorocyclohexane (HCH), the DTH response at postnatal day 10 was significantly increased in mice exposed to 10 mg/kg HCH and significantly impaired in mice exposed to 100 mg/kg HCH (*53*). Spleen cell responses to concanavalin A (Con A) and lipopolysaccharide (LPS) were 2- and 8-fold higher, respectively, and the number of antibody plaque-forming cells (PFC) was 2-fold higher relative to control values in pups from the 10 mg/kg group, whereas neither mitogen nor the PFC responses were altered by 100 mg/kg HCH. Immunostimulation has been described at certain (usually low) dose levels of chemicals that are otherwise immunosuppressive, although the biological significance is unknown (*57–59*).

Selective and highly persistent immune alterations have also been observed in mice after gestational exposure to the organochlorine insecticide chlordane. Mice exposed to chlordane during fetal life display significant depression of cell-mediated immunity that is still present 101 days after birth, reduced numbers of granulocyte-macrophage colony-forming units and colony-forming units in the spleen at 200 days of age, and long-term depression of both DTH and mixed lymphocyte reactivity (*55,60*). It is noteworthy that these immune effects are either reduced or not observed in adult mice exposed to chlordane at dose levels equal to those given to the pregnant mice.

PAHs. Immunosuppressive PAHs [e.g., B[*a*]P, methylcholanthrene, and dimethylbenz[*a*]anthracene] have become ubiquitous environmental contaminants as a result of their occurrence in petroleum, coal and coal tars, soot, air pollutants, tobacco smoke, and cutting oils (*61–63*). The ability of certain PAHs to cross the placenta (*64*) as well as to produce immunosuppression in adult animals raised questions about the intensity and persistence of effects of these agents on the developing immune system. Urso and Gengozian (*65,66*) and Urso and Johnson (*67*) reported severely depressed humoral immune function in mice exposed to B[*a*]P from days 13–17 of gestation. In the latter of these reports (*67*), these authors also observed highly persistent (still present at 18 months of age) impairment of cell-mediated immunity in offspring in the form of suppressed mixed-lymphocyte and graft-versus-host responses. Increased tumor frequency was further observed in the mice exposed to B[*a*]P before birth, suggesting the lesion to the developing immune system may provide a favorable environment for the growth of neoplasms.

The toxicity of B[*a*]P is in part due to production of a highly reactive epoxide metabolite by microsomal mixed-function oxidase enzymes that covalently bind DNA and other nucleophilic intracellular macromolecules. Thus, rapidly proliferating cells, such as those composing the immune system, are targeted (*68*). Interestingly, a study evaluating the covalent binding of B[*a*]P in fetal mouse tissues found that liver hematopoietic cells were the most active (*69*). Further, the extent of transplacental enzyme induction compared to control was greatest in hematopoietic cells (18-fold), followed closely by whole fetal liver (16-fold) (*67,69*). Such results indicate that fetal liver, the primary hematopoietic organ in the fetus (*4,6–8*), and its hematopoietic cells are specific targets of B[*a*]P, and may explain, at least in part, the significant toxicity to the developing immune system resulting from exposure to this compound. In addition to covalent interaction with DNA, PAHs also bind the *Ah* receptor and thus may cause TCDD-like immune effects through noncovalent interactions of the receptor–ligand complex with DNA. The observation of a TCDD-like inhibition of thymocyte differentiation in day-18 fetal mice exposed to TCDD from days 13–17 of gestation may suggest *Ah*-mediated immunotoxicity from PAHs (*70*). In addition to inhibited thymocyte maturation, these authors reported severe fetal thymic atrophy and thymocyte depletion and decreased fetal liver hematopoietic cell counts. Prolymphoid cells (both T and B precursors), identified by cell surface antigen expression and by the presence of terminal deoxynucleotidyl transferase, were also significantly decreased. These authors hypothesized that decreased numbers of fetal liver prothymocytes may contribute to fetal thymic atrophy via reduced colonization of the thymus, and that reduced pre-B cells may relate to the depressed humoral immunity caused by prenatal exposure to B[*a*]P. Similar effects on the development of immunity in mice exposed to 7,12-dimethylbenzanthracene have been reported (*71*).

Steroid hormones (estrogen). An altered prenatal hormonal environment has been associated with changes in postnatal immune function in mice (*72*). However, the role of sex steroids in the development of the immune system remains poorly understood at best. Reports in the literature indicate that exogenous androgenic (*73*) or estrogenic compounds (*74–81*) can alter normal development of the immune system. The available data largely report effects of estrogen and estrogenic compounds (e.g., zearalenone and the nonsteroidal estrogen DES). Exposure to pharmacologic or suprapharmacologic levels of either steroidal or nonsteroidal estrogens results in numerous alterations of immune function, particularly when administered perinatally. The effects of such administration in rodents include myelotoxicity (*82,83*), suppression of cell-mediated immunity (*84*), pronounced thymic atrophy (*85,86*), depression of NK cell activity (*87,88*), and stimulation of the reticuloendothelial system (*81,89*). Studies examining postnatal immune function in humans exposed *in utero* to exogenous estrogen are limited to a few retrospective evaluations of adults exposed during pregnancy to DES. These currently unconfirmed reports suggest possible adverse immune effects in humans, including altered T-lymphocyte and NK cell function in adulthood (*76,90*) and increased incidence of autoimmune diseases (*74,91*).

The available rodent data support the indication in humans that developmental exposure to DES may alter postnatal immunocompetence. Long-term impairment of both humoral (antibody production), innate (i.e., NK cell activity), and cell-mediated (T lymphocyte mitogen responsiveness, graft-versus-host reaction, and delayed hypersensitivity) immune function has been associated with perinatal DES exposure in mice (*75–78*). Depression of these functional assays has further been associated with increased postnatal susceptibility to viral-induced mammary tumors and to transplanted primary and carcinogen-induced tumors (*79,80*). It has been suggested that a possible relationship may exist between prenatal DES exposure and neoplastic growth in humans, including clear-cell adenocarcinoma of the vagina (*92,93*).

Lead. Administration of lead throughout gestation and lactation in rats resulted in reduced thymic weights and inhibited DTH and lymphoproliferative responses at 35–45 days of age (*45*). Lead exposure in both rats and chickens during early development also caused persistent shifts in humoral immune function, apparently as a result of effects on T-helper cells (*94*). It is important to note that the dose levels of lead used in these studies did not cause immunologic effects in adult rats. Bunn et al. (*95*) reported altered Th1-/Th2-associated functions in female but not male rats after exposure to lead during development. Further, the lack of maternal influence in the developing chicken model used by these authors suggests a direct immunomodulatory effect of lead on the developing immune system, at least in this species. Chen et al. (*96*) also suggested that there are periods of greater sensitivity to impairment by lead exposure during rodent development.

Therapeutic agents. Many chemotherapeutic agents (e.g., alkylating antineoplastic drugs such as cyclophosphamide or chlorambucil) are potent immunosuppressants simply because they are powerful antiproliferative agents, and many aspects of a functioning immune system require active proliferation. Other drugs such as therapeutic immunosuppressants are specifically administered for their immunotoxicity, to control autoimmune and hypersensitivity diseases, to control certain inflammatory conditions, or to prevent organ transplant rejection. Human pregnancy can exacerbate autoimmune disease, requiring therapeutic intervention during this critical time. Further, recent advances in organ transplant success have resulted in increasing numbers of women becoming pregnant after an organ or tissue (allograft) transplant procedure. These women require therapeutic immunosuppressants throughout pregnancy to prevent allograft rejection. Thus the probability of immunotoxic drug exposure during pregnancy may in some cases be increasing. In other instances, women who are unaware they are pregnant may be

exposed to a wide variety of potentially immunotoxic therapeutic regimens.

The antileukemic alkylating agent busulfan caused severe thymic hypoplasia in mice treated with a single dose during midgestation (*97*), raising questions about the similar effects of other therapeutic alkylating agents. Cyclophosphamide has been widely used as an antineoplastic agent and, previously, as an immunosuppressant both after organ transplant and for autoimmune disease control (*98,99*). Further, cyclophosphamide treatment in adults is particularly toxic to B lymphocytes (*100,101*) and results in impaired antibody production to T-dependent and T-independent antigens (*102,103*), decreased lymphoproliferative responses (*103*), impaired NK cell activity (*104*), and impaired host resistance to infectious agents and syngeneic tumor cells (*105*). Chickens exposed to cyclophosphamide during embryonic/neonatal development displayed near-total eradication of the lymphocyte population within the bursa of Fabricius, and complete suppression of humoral immunity (*106,107*). However, studies in pregnant mice found no differences in spleen or thymus weight or histology, hematological profiles, antibody responses, or DTH responses, even at doses of cyclophosphamide that significantly decreased fetal body weight (*108*). Cyclophosphamide is metabolized to an active alkylating agent, and both the parent compound and the active metabolite cross the placenta (*109*). Fetal metabolism of cyclophosphamide does not appear to occur, however; thus, teratogenic or fetotoxic doses of this drug may be required before immunotoxic levels of metabolite are formed in the fetus (*108*).

In the past, glucocorticoid hormones and cytotoxic (chemotherapeutic) drugs were widely used to inhibit tissue allograft rejection; however, prolonged use and/or use of larger doses of these drugs was severely limited by numerous, and in many cases serious, side effects (*110*). Thus, considerable research effort has been directed toward the development of new and more effective immunosuppressive drugs. The potent immunosuppressant, cyclosporin A (CsA), was introduced into clinical trials in 1978 (*111*) as a result of such efforts, and since has become the most widely used agent for the prevention of human organ transplant rejection (*112–114*). CsA is now considered the major reason for the present success of organ transplant surgery (*115*). Effective therapeutic immunosuppression with CsA, however, often requires simultaneous administration of additional immunosuppressive drugs [e.g., azathioprine (AZA), 15-deoxyspergualin, mizoribine, didemnin B, and corticosteroids, including prednisolone (*116–123*)]. More powerful and less toxic antirejection drugs are still highly desired and are currently being developed and tested.

Numerous successful births have been reported in women receiving potent immunosuppressants throughout pregnancy, including CsA (*117,118,124–126*), AZA (*117,118,127,128*), and prednisolone (*118,129,130*). Limited amounts of CsA or AZA cross the placenta; however, observations of transient neonatal thrombocytopenia, leukopenia, and hypoplasia of the lymphatic system in children exposed to CsA (*124*) or to AZA and prednisolone (*131,132*) during pregnancy suggest that some developmental immunotoxicity results from maternal exposure to these agents. The long-term immune consequences (e.g., postpuberty) from such exposure in these children remains unknown.

Compared to AZA, CsA treatment during human pregnancy carries a higher risk of premature delivery as well as for the delivery of small-for-gestational-age (SGA) babies. In a recent study of 238 women receiving a variety of immunosuppressive drug regimens during pregnancy, the rates for both prematurity and SGA infants were high (49 and 29%, respectively) (*133*). Regimens that included CsA resulted in 66% prematurity (43% with AZA) and 56% SGA babies (19% with AZA). However, data from this study indicated, in contrast to previous reports, that AZA may carry a higher risk of congenital malformation than CsA. Birth defects presently associated with CsA administration during pregnancy include cataracts (*116,134*), dysmorphic facial appearance (*135*), cleft palate (*136*), aseptical necrosis of the femoral head, and additional fetal malformations induced by recurrent viral infection (*137*). The resultant uncertainty surrounding the potential of these agents to induce developmental malformations in humans, as well as regarding "safe" levels of these agents to be administered during pregnancy, was underscored by the results of a recent survey of pediatric gastroenterologists in which the "vast majority of responders were not certain what to recommend with respect to the use of immunosuppressive agents prior to and during pregnancy" (*117*). This remains true largely because the primary clinical objective of such immunosuppressive therapy, until quite recently, has been maintaining the life of the recipient rather than allowing recipient individuals to conceive and bear children. It may be important that these studies did not include neonatal immune end points as a focus, especially with the consideration that the immune system is the target organ of the drugs being administered.

Rodent studies that use fetal thymic organ culture techniques demonstrated that CsA exposure alters early development of thymocytes within the fetal thymus, completely blocking the generation of mature T cells (*112*), indicating that the fetal immune system may be targeted by maternal dosing with CsA. Because low levels of CsA cross both the rat (*138*) and human (*133,139,140*) placenta, it is reasonable to anticipate that CsA may affect the same target organs in the embryo as in the adult. Further, laboratory mice exposed perinatally to CsA displayed hypoplastic peripheral lymphatic organs, impaired intrathymic thymocyte differentiation, absence of mature T cells in lymph nodes and spleens, and lack of functional T-cell reactivity (*141*). However, in these studies mice were exposed to CsA only during the third trimester of pregnancy, with continued exposure for as long as 28 days postnatally. Thus, the consequences of earlier prenatal exposure, as well as the contribution of prenatal exposure alone, to immune alterations were not determined.

CsA also interferes with tolerization of developing T lymphocytes after syngeneic bone-marrow reconstitution, resulting in autoimmunity (i.e., syngeneic graft-vs.-host disease) in the host animals (*142,143*). Such results raise questions about the ability of CsA to induce or exacerbate autoimmune disease in gestationally exposed individuals. Zacharchuk et al. (*144*) recently found that the susceptibility of thymocytes to clonal deletion changes during ontogeny. Studies by these authors indicate that there is a relatively synchronous wave of maturing thymocytes that are susceptible to deletion signals during fetal life and shortly after birth, but not 7 days after birth. Such an observation is in agreement with the current understanding of neonatal tolerance and further suggests that failure to induce tolerization in glucocorticoid-exposed 1-week-old mice reflects an alteration in susceptibility to normal clonal deletion during that time (*144*). Indeed, a recent study of newborn mice dosed daily with CsA during the first week of postnatal life demonstrated the subsequent development of organ-specific autoimmune disease (*145*). For such reasons, ablactation has been recommended in women treated with CsA during pregnancy because of the transfer of CsA equivalent to 5% of an immunosuppressive dose into the breast milk, and because of the potential toxicity and unknown side effects of CsA on the child's immunologic system (*146*). Of perhaps greater importance, it has now been determined that the blood of such newborns contains CsA concentrations as high as 65–85% of maternal levels (*147*). Taken together, these studies raise questions regarding the ability of transplacental CsA exposure to contribute to the expression of autoimmune disease later in the life of gestationally exposed individuals.

The use of therapeutic immunosuppressants during pregnancy thus represents a relatively new area of human developmental exposure to immunotoxic agents. In some regards, the effect of these drugs on the developing immune system have largely been disregarded in the assessment of risk to the unborn despite the known heightened sensitivity of the immune system to chemical insult during development.

Prenatal chemical exposure and postnatal autoimmune disease. The observation that CsA treatment in irradiated rodents leads to expression of autoimmune disease after bone marrow transplantation raises questions about other chemical agents that might have this effect. TCDD produces fetal thymic effects *in vivo* similar to the effects of CsA in fetal thymic organ culture, including inhibited thymocyte maturation and reduced expression of thymic MHC class II molecules. These observations led to the suggestion that gestational exposure to TCDD may interfere with normal development of self tolerance [reviewed by Holladay (*148*)]. It has also been suggested that humans exposed to DES during immune development may display increased incidence of autoimmune disease. A retrospective study of women (1,711 individuals) exposed to DES during pregnancy found that the overall frequency of any autoimmune disease was 28.6/1,000 compared to 16.3/1,000 among unexposed controls (significantly different at $p = 0.02$) (*74*). The autoimmune diseases evaluated included systemic lupus erythematosus, scleroderma, Grave disease, Hashimoto thyroiditis, pernicious anemia, myasthenia gravis, thrombocytopenic purpura, rheumatoid arthritis, regional enteritis, chronic ulcerative colitis, multiple sclerosis, chronic lymphocytic thyroiditis, Reiter syndrome, and optic neuritis. When these autoimmune diseases were considered individually, however, only Hashimoto thyroiditis occurred significantly more often in the exposed women ($p = 0.04$). A similar evaluation of 1,173 humans exposed to DES during development (1,079 females and 94 males) found increased asthma, arthritis, and diabetes mellitus compared to prevalence rates for these diseases in the general population (*91*). However, in a more recent study evaluating rates of allergy, infection, and autoimmune disease in DES-exposed individuals (253 men and 296 women) matched with similar unexposed individuals (241 men and 246 women), no differences in disease occurrence were detected (*149*). Baird et al. (*149*) concluded that a larger sample was needed to evaluate DES-associated risk of autoimmunity because autoimmune diseases are relatively rare in the human population. Thus, preliminary studies of humans exposed before birth to DES suggest the possibility of postnatal immune alterations, including increased autoimmune disease.

Rodent studies that evaluated increased expression of autoimmune disease after developmental immunotoxicant exposure have been limited. However, a recent report indicates that mice predisposed to the development of autoimmune disease display exacerbated disease if exposed to DES or TCDD during gestation. Silverstone et al. (*150*) found that a single fetal exposure to TCDD in NZB × SWR (SNF1) mice significantly reduced the time to postnatal onset of autoimmune lupuslike nephritis in the male offspring. These authors reported a similar observation for DES, i.e., a single fetal exposure of SNF1 mice to DES induced autoimmune nephritis in male offspring between 5 and 10 months of age. Female SNF1 mice develop this autoimmune syndrome spontaneously during the first year of life; however, male mice do not display significant autoimmunity before 1 year of age. This report suggests that prenatal exposure to certain immunotoxicants may play a role in postnatal expression of autoimmunity.

Postnatal Immunotoxicant Exposure

There are few developmental studies in which experimental animals have been exposed to immunotoxicants only during the postnatal period.

Although it is not an immunotoxic agent, malnutrition during early childhood adversely affects the immune system. Dietary protein deprivation in malnourished young children between 1 and 3 years of age resulted in a number of immune deficiencies. These included decreased IgG and trends toward lower IgA and IgM levels, decreased cutaneous hypersensitivity responses, decreased T lymphocyte responses to phytohemagglutinin (PHA), and reduced responses to *Salmonella typhi* vaccine (*151*). Four- to six-week-old mice fed a low protein diet displayed decreased T cells and decreased responses to PHA and allogeneic cells in the mixed-lymphocyte response (MLR), as well as retardation of immune maturation (*152*). Zinc deficiency in the diet of lactating mouse dams resulted in pups that displayed retardation of thymus and spleen development, depressed T-cell mitogen responses, and the absence of detectable IgM and IgG (*153–155*).

The administration of certain drugs during early postnatal life can also result in altered immune function later in life. For example, 1-day-old mice and rats exposed to cortisol acetate or hydrocortisone, respectively, displayed thymic atrophy and reduced antibody responses to sheep red blood cells (SRBC) (*156,157*). When given to 1- to 5-day-old mice, DES, a nonsteroidal drug having estrogenic activity, resulted in a number of immune abnormalities. These abnormalities included reduced T- and B-cell mitogen responses to PHA and LPS, respectively; reduced antibody responses to SRBC and LPS; reduced T-helper cells; and reduced NK cell activity (*88,158,159*).

Toxic chemicals administered to young animals may also alter immune function, which may be more severe than that in adults or which may persist for a longer period of time. For example, the exposure of rat pups to di-*n*-octyltin dichloride during the first 3 weeks of life resulted in suppression of the PHA response, which persisted up to 10 weeks of age (*160*). Rat pups exposed to cocaine from postnatal day 1–21 had decreased peripheral blood lymphocytes on day 22, whereas pups exposed to both cocaine and ethanol had decreased total IgM levels (*161*). Weanling (i.e., 25-day-old) rats dosed 4 times over 17 days with 3,3′,4,4′-tetrachloroazoxybenzene had reduced thymus weights, antibody responses to SRBC, peritoneal macrophage chemiluminescence, and bone marrow cellularity. These effects were greater in rats exposed as weanlings compared to adults (i.e., 56 days old at the start of dosing) (*52*).

Comparison of Similarities and Differences Between Humans and Laboratory Animals

Direct comparison of immune development between human and animals is complicated by several factors. However, the most significant difference lies in the developmental maturity of the immune system before and after parturition. This difference has been linked to the length of gestation. For example, animals with short gestation periods (e.g., mice, rats, rabbits, and hamsters) have relatively immature immune systems at birth compared to humans, with significant immune development occurring postpartum. Nevertheless, there are more similarities than differences in the ontogeny of the human compared to laboratory animal immune systems. In fact, a significant amount of our knowledge about human immune system development has been achieved because of animal research.

To present the similarities and differences in the ontogeny of immune system cells for which there are data in both humans and mice, we used a convention to compare prenatal immune system maturational landmarks between the species. The period of gestation for the mouse is approximately 20 days; that for the human is 40 weeks. Therefore, each day of gestation for a mouse is approximately equivalent to 2 weeks of gestation for the human. We expressed the time of gestational

development for each maturational landmark for which there are comparable data for the human and the mouse, as a decimal portion (dp) of the respective gestational periods (Table 1). For example, if a specific maturational landmark occurs on week 10 for the human and on day 12 for the mouse, the dp would be 10/40 = 0.25 for the human and 12/20 = 0.60 for the mouse.

Embryonic hematopoietic stem cells in the fetal yolk sac begin to migrate in the human fetus at approximately 5 weeks (dp = 0.125), which results in the presence of lymphocyte precursors in the liver by 7 weeks (dp = 0.175) and in the thymus after 9 weeks (dp = 0.225) (*162*). At 10 weeks (dp = 0.25) the fetal thymocytes express the T-cell markers CD3, CD4, CD5, and CD8 (*163*). Lymphocytes begin to appear in peripheral blood shortly after their appearance in the thymus and then in the spleen (*164*). In the mouse, the thymic epithelial anlage is colonized by bone marrow-derived stem cells on approximately gestation day 11 (dp = 0.55). By day 14 (dp = 0.70), murine thymocytes express CD4 and CD8 (*165*). For these maturational landmarks the mouse is chronologically 2- to almost 3-fold more immature than the human, respectively.

PHA-responsive lymphocytes in the human fetal thymus are detectable by 12 weeks (dp = 0.3), with splenic and peripheral blood lymphocyte responsiveness to PHA detectable approximately 4 weeks later (dp = 0.4) (*166*). Responses to Con A have been observed with thymus cells at 13–14 weeks of gestation (dp = 0.35) and with spleen cells at approximately 18 weeks of gestation (dp = 0.45) (*167*). At 11–15 weeks of gestation (dp = 0.275–0.375), lymphocytes from human fetuses are capable of stimulating and responding to allogeneic lymphocytes in an MLR (*168*). On the other hand, mouse thymocytes only begin to respond to PHA, Con A, and in an MLR by gestation day 17 (dp = 0.85), which is greater than a 2-fold delay compared to the human. Although the levels of thymocyte responsiveness to PHA and in the MLR in mice are similar to adult levels, Con A responsiveness does not reach adult levels until 2–3 weeks after birth (*169*).

Table 1. Time of gestational development (dp).

Maturational landmarks	Human	Mouse
sIg expression on B cells	0.10–0.15	0.85
Fetal liver hematopoiesis first detected	0.15	0.50
Lymphocyte precursors in thymus	0.23	0.55
Mitogen responsive hymocytes	0.35	0.85
Functional NK cells	0.35	> 1.0
Pluripotent stem cells seed bone marrow	0.50	0.85

The development of functional NK cells in the human fetus occurs at 28 weeks of gestation (dp = 0.35), with full-term newborns displaying peripheral blood NK activity at approximately 60% that of adult levels (*170*). In contrast, NK cell activity in mice is absent at birth and does not begin to appear until about 3 weeks of age (*171*).

The development of B lymphocytes in humans begins at approximately the same time as T lymphocytes. B lymphocytes (i.e., lymphocytes bearing sIgM and sIgG) are first found in the liver at about 8 weeks of gestation (dp = 0.10) and in the spleen at about 12 weeks (dp = 0.15). IgD- and IgA-bearing cells are found at 12 weeks of gestation (dp = 0.15). Adult levels of B lymphocytes bearing sIg of all classes are reached by 14–15 weeks of gestation (dp = 0.35) (*172*). However, human neonatal B lymphocytes are functionally defective in their capacity to generate antibody-producing cells *in vitro* compared to B cells from adults. In general, B lymphocytes of humans are inherently immature at birth. Although a small number of IgM-producing cells are detected, no IgG- or IgA-producing cells can be identified. The ability of B cells to produce antibodies of the IgG and IgA classes increases with age, with adult levels reached by 5 and 12 years of age, respectively (*173*). In fetal mice, B cells expressing sIg appear in the liver, spleen, and bone marrow on day 17 (dp = 0.85) (*6*), which represents an approximate 3-fold delay compared to the human. In mice, antibody responses to T-cell-independent antigens occur soon after birth and reach near-adult levels by 2 or 3 weeks. In contrast, T-cell-dependent antibody responses in mice begin after 2 weeks and do not reach adult levels until 6–8 weeks of age (*174*).

It is not clear why there are such delays in B lymphocyte function. Deficiencies in IgG and IgA responses during the early period of life have been described as reflecting regulation by T cells (suppressor cells) as opposed to a deficiency in B cells. This high-suppressor T-cell activity has been reported to exist throughout infancy with decreasing intensity and disappears by approximately 2 years of age or later (*173*). On the other hand, a case has been made that deficient B-cell function during the first several years of life is due to a B-cell defect or immaturity rather than to a negative influence by T-suppressor cells or monocytes (*172,175*). In any event, it has been postulated that the greater susceptibility of human newborns to certain bacterial infections is due to deficient B-cell function, particularly because of the delay in production of IgG and IgA antibodies (*172*). Infants appear to respond well to T-cell-dependent antigens primarily through adequate production of IgM. However, they respond poorly or not at all to antigens such as the polysaccharide antigens found on the cell walls of several infectious bacteria, which also contributes to increased susceptibility to infections (*175*). It is interesting to note the dichotomy between humans and mice in the maturation of antibody responses to T-cell-dependent and -independent antigens.

The immune responsiveness of human neonates appears to be influenced by their sensitization to antigens *in utero* and during early postnatal life. There is ample evidence from laboratory studies to demonstrate that when animals are raised from birth in a germ-free environment there is a delay in lymphoid development (*176*). Germ-free animals have significantly reduced levels of Ig, which is due to a 2–5 times lower rate of Ig synthesis. Synthesis of IgA and IgM is more affected than IgG synthesis in a germ-free environment. The number of plasma cells in these animals is approximately 10 times lower than in normal animals (*172*). Further, germ-free animals have a difficult time replacing maternal IgG, which is transported during gestation across the placenta, with a sufficient production of IgG of their own.

At birth nearly all of the IgG in the newborn human is of maternal origin. During the first 3–6 weeks of life, there is an exponential decrease in IgG that reaches its lowest level between 1.5 and 3 months of age, when IgG synthesis by the newborn begins; however, adult IgG levels are not reached until 5–6 years of age. On the other hand, IgM levels rise rapidly during the early months of life, with IgM at 75% of adult levels within 12 months of life. Only 20% of adult IgA levels are present at 1 year; adult levels are reached only after 10 years of age (*177*). The early increase in IgM levels in newborns parallels the development of natural antibodies to enterobacteria. Studies with humans indicate that exposure to various nonpathogenic microorganisms or vaccines early in life gives rise to higher serum Ig titers than seen in noninfected/nonimmunized infants (*177*).

Human fetal macrophages originate from hemopoietic stem cells and appear in tissues through the circulation after vascularization. In mice, immature macrophages can be found in the yolk sac at approximately gestation day 10–11 and then in other tissues and in circulation at day 12 or later (*178*). Although fetal macrophages generally lack the ability to kill certain bacteria after phagocytosis, neonatal rodents possess bactericidal activity, albeit less than that of adults (*179*). On the other hand, the phagocytic and antitumor activities of newborn mouse macrophages are higher than those of adult mice (*178*).

The functional immaturity of leukocytes is a significant factor in the human neonate's

impaired host defenses (*180*). The neonate's inflammatory response is insignificant compared to that of the adult. This decrease in inflammatory responsiveness in the neonate is characterized by a higher proportion of functionally immature polymorphonuclear neutrophils (PMNs) and fewer monocytes during the early hours of inflammation compared to infants 3–5 months of age. During the early period of inflammation, a large number of eosinophils are observed, which is unique to this early period of life (*181*). PMNs and monocytes from neonates (i.e., cord blood) are deficient in their chemotactic responses compared to cells from adults (*180,182*). Variable results have been obtained in studies on the bactericidal activity of PMNs from newborn humans, but in the absence of unusual circumstances this activity appears to be normal (*180,182*). After infection, the pool of PMN and PMN precursors in the newborn animal is rapidly depleted compared to adults (*183*). Furthermore, the number and rate at which PMNs reach the site of infection in newborn rats are decreased compared to adults (*184*).

Complement components and activity have been studied during fetal and neonatal life in humans. The complement component C3 appears in fetal tissues as early as 6 weeks of gestation, with C2 and C4 detected at 8 weeks of gestation (*185*). The appearance of complement in fetal tissue is due to synthesis by the fetus because little or no transfer of complement occurs across the placenta (*186*). In general, it appears that many of the components of complement, as well as the hemolytic activity of complement, are significantly lower in newborns compared to adult levels. Maturation of components of the classical and alternative complement pathways occurs in an age-related fashion, with C2, C4, C5, C6, and B reaching adult levels by 6 months of age (*187*). Although these quantitative deficiencies are not necessarily associated with functional defects, the increase in complement proteins during the first 6 months of life may partially explain the age-restricted susceptibility to bacterial infection (*188*). In fact, evidence suggests that deficiencies in the opsonizing capacity of complement in neonatal serum may contribute to this susceptibility (*180*). Work in fetal sheep and pigs has demonstrated that whole complement activity in serum is not detectable until after day 123 of gestation in the sheep (normal gestation of 145 days) and that in pigs the levels of C8 and C9 are only 30% of adult levels at term (*189*).

The *in vitro* induction of interferon production in lymphocytes obtained from fetal (8–17 weeks of gestation), newborn, child (1 month to 10 years), and adult tissue have been studied. The interferon titers were in general the lowest among the fetal tissue and the highest among newborns and children. However, the data suggested that the interferon-producing capacity of human lymphocytes remains relatively constant throughout intrauterine and postnatal life (*186*).

Gaps in Knowledge

There are several gaps in the present knowledge base of drug- and chemical-induced developmental immunotoxicity in humans and animals. For example, although it is known when certain structural components of the immune system first appear and become functional during development, comparative information regarding the outcome of exposure to chemical agents or mixtures of chemicals before, during, or shortly after any given aspect of immune development is largely unavailable. As a case in point, it is not known if exposure to TCDD before, during, or after the establishment of the thymic rudiment contributes most to the postnatal immunosuppression caused by this chemical. Such is true for essentially any immunotoxicant one may wish to consider.

Continued animal studies remain the most obvious way to define the most sensitive periods of immune system development to immunotoxicant exposure. In this regard, although there are numerous studies in laboratory rodents that have demonstrated the sensitivity of immune system development to perturbation by physical or chemical insults, many of these studies used both pre- and postnatal exposure regimens. Thus, it is often unclear which portion of such exposure contributes most to subsequent immune impairment. A first step in making this determination would involve cross-fostering studies with known developmental immunotoxicants to determine and compare the effects of *in utero* versus lactational and/or early postnatal exposure. In addition, the development and application of more sensitive and predictive end points of developmental immunotoxicity would facilitate the identification of critical windows of exposure.

Another gap in present knowledge is the role that toxicokinetic and/or toxicodynamic mechanisms play in age-dependent responses. Specifically, it is uncertain if the inherent increased susceptibility of the young animal/human to immunotoxicants is due to reduced ability to metabolize and/or excrete the toxic moiety, or is more the result of the exposure occurring during critical developmental phases that require waves of rapid cellular proliferation and differentiation. Kinetic studies and studies evaluating immunotoxicant effects on the genes regulating cell cycle and apoptosis in developing immune tissues are needed to begin to answer these questions.

Information regarding possible consequences of exposure to immunotoxicants very early in gestation (e.g., postconception/preimplantation) is also essentially unavailable. For instance, it is presently not known if certain chemical agents may affect hematopoietic progenitor cells in the yolk sac before migration to fetal liver, affecting immune development. Similarly, no information is available regarding possible adult exposures (i.e., preconception) that may have the potential to alter the development of the fetal immune system. The National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences has, over the past 15 years, developed a two-generation study design (i.e., reproductive assessment by continuous breeding) specifically aimed at identifying potential hazards to male and/or female reproductive performance resulting from exposure to toxic chemicals (*190*). This NTP paradigm for detecting reproductive impairment in the offspring of rodents in which the male and/or the female parent was exposed to chemical agents before and/or throughout the breeding and lactating period could be directly applied to studying effects of parental chemical exposure on immune development of the offspring.

An additional recent area of concern in developmental immunotoxicity is that early-life exposure to certain chemical agents may not only produce postnatal immunosuppression but also contribute to increased expression of hypersensitivity responses or autoimmune diseases later in life. A careful evaluation of this possibility that gestational exposure to certain immunotoxicants may relate to postnatal expression of aberrant immune function is needed and represents a major gap in our present understanding of the possible consequences of immunotoxicant exposure during development. Although rodent studies will be critical to providing data to fill this gap, recent success in organ transplant procedures is resulting in a cohort of humans who were exposed to therapeutic immunosuppressive drugs from the preconception period throughout gestation. These individuals offer an unusual opportunity to expand the human developmental immunotoxicity database, and should be carefully followed in clinical and epidemiologic studies.

Summary

Experimental studies suggest that the production of long-term secondary immunodeficiency in adult animals requires either continued exposure to the inducing agent or damage to primitive hematopoietic cells. In contrast, low-level single-dose exposure to certain immunotoxicants during immune development may produce changes in the immune system that are long-lasting or

permanent, and that may be irreversible. Thus, for at least some immunotoxic assaults, it seems clear that the developing immune system is highly sensitive relative to adults, and, therefore, that developmental immunotoxicant exposure should be limited as much as possible.

It must further be emphasized that, to date, postnatal immune end points evaluated in experimental animals exposed during gestation to immunotoxicants have focused almost exclusively on inhibited immune function rather than on aberrant immune responses. Clearly, the possibility that developmental exposure to immunotoxicants may play a role in inducing or exacerbating hypersensitivity or autoimmune responses needs to be investigated in laboratory animal studies. Further clinical and epidemiological studies using large well-defined cohorts will also need to be undertaken to establish whether acute or chronic low-level exposure during prenatal life contributes to human immunologic dysfunction and subsequent clinical disease.

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